REMARKS

The Examiner objected to Claim 1 because of an informality. The Claim has been amended as shown above to cure this defect and also to correct other minor typographical errors.

Claims 1-4, 7-15, 19, and 20 were rejected under 35 U.S.C. 103(a) as being unpatentable over Bohn *et al.* (US 7,220,345 B2) ("Bohn") in view of Deamer (US 6,428,959 B1) ("Deamer") and Su *et al.* (US 7,005,264 B2) ("Su"). Applicant submits that these claims as currently amended are not obvious in view of the cited prior art.

Claim 1 requires a set of electrodes in each of the two channels. The Examiner maintains that although Bohn does not disclose providing a second set of electrodes in the second channel, it would have been obvious to do so in the system taught by Bohn "because this will allow the molecules to undergo another separation and so further resolve the different types of molecules from each other. Indeed, Bohn implicitly contemplates this as "... a high resolution second-dimensional separation" is disclosed. See col. 06:62-66."

Claim 1 has been rewritten to make it clear that the electrodes are disposed within the second channel on opposite sides of the nanopore. As explained in the specification of the current invention (Page 11, lines 24 – 29) the purpose and function of the set of electrodes in the second channel is to create the electric field that draws the biopolymer, headed by its leader molecule, through the nanopore from the first channel into the second channel. As discussed further (Page 15, lines 17-29) the electric field set up in the second channel can be controlled independently of the field set up in the first channel, and the two fields can be adjusted to manipulate the biopolymer as it traverses the nanopore, stretching or compressing the biopolymer as required in the course of the sequencing operation which is the larger purpose driving this invention. Applicant submits that the use of multiple electrodes, disposed on either side of the nanopore in the second channel, is particularly advantageous in allowing the electric potential at the base of the nanopore to be set and maintained at a desired value, or adjusted as necessary, independent of the significant changes in the resistance of the nanopore as the

biopolymer passes through it.

The Examiner suggests that one would be motivated to add a second electrode to the system taught by Bohn to allow for a second electrophoretic separation to occur in the second channel. Applicant submits that electrophoretic motion of molecules passing through the pores in the system taught by Bohn must occur anyway, under the influence of the field that must exist in the second channel to cause the passage through the pores. Hence, Applicant submits that there would be no need to add a second electrode to the system taught by Bohn to achieve electrophoretic separation in the second channel. Moreover, even if one were to make that addition, there would be no reason to position that second electrode on the opposite side of the nanopore in the second channel to the position of the first electrode in the second channel.

Accordingly, Applicant submits that Claim 1 and the Claims dependent therefrom are not obvious in view of the cited prior art.

Claim 2 requires a substrate having a channel wall and a nanopore in the channel wall. The Examiner points to Bohn as teaching a nanopore (42) in the wall of the first microfluidic channel (Figures 1 and 5A-5C and col. 01:58-63 and claim 3). Applicant submits that the nanopore taught by Bohn exists within a membrane 22 separate from the elements containing the channels. Hence, the nanopore taught by Bohn is not present in a channel wall that exists within a substrate, as required by the Claim. Deamer and Su do not provide the missing teachings. Hence, Applicant submits that Claim 2 is not obvious in view of the cited art.

Claim 3 requires at least one set of electrodes for moving the biopolymer in a second direction through the nanopore after the biopolymer has been moved past the nanopore. The Examiner maintains that although Bohn does not disclose providing a second set of electrodes in the second channel taught by Bohn, it would have been obvious to do so "because this will allow the molecules to undergo another separation and so further resolve the different types of molecules from each other. Indeed, Bohn implicitly contemplates this as "... a high resolution second-dimensional separation" is disclosed. See col.

06:62-66."

First, as noted with respect to the rejection of Claim 2, the cited art does not teach a nanopore in the wall of a substrate.

Second, the Examiner has not pointed to any teaching that the electrodes are disposed such that the biopolymer moves past the nanopore prior to being drawn through the nanopore. The Examiner attempts to overcome this problem by arguing that statistically some of biopolymers must move past the nanopore prior to being drawn into the nanopore. However, the Examiner provides no support for this speculation. Hence, Applicant submits that Claim 3 and the claims dependent therefrom are not anticipated by the cited art.

Claim 14 depends from Claim 1 and further requires that the first channel diameter is from 1 to 10 micrometers. The Examiner states that "Bohn only mentions a channel width of 100 micrometers and a depth of 30 micrometers as example dimensions" The Examiner maintains that "Barring a contrary showing the claimed channel dimensions are just a matter of scaling the channel for the expected volume of fluid to be contained by the channel, which will be proportional to the expected sample volume." Applicant submits that to the contrary, the specific dimensional range specified by Claim 14 is not merely a matter of scaling for the expected fluid volume, but is chosen with regard to other significant criteria.

Specifically, as the electric field drawing the biopolymer of interest through the nanopore is only experienced in the near vicinity of the pore itself, almost all of the fluid present across the diameter of a channel is orders of magnitude greater than the pore diameter that would pass that pore without experiencing that field. Hence, there is a significant advantage in limiting the channel diameter to values large enough to be easily fabricated but small enough to avoid wasting fluid that is passed through the device but not subjected to that second field.

Hence, Applicant submits that there are additional grounds for allowing Claim 14.

With respect to Claim 15, the dimension in question sets the length of the nanopore. If the length is small compared to the length of the biopolymer, the biopolymer will not be threaded through the nanopore in a manner that allows for its detection as a "stretched out" molecule. If the length is too long, the electric fields needed to provide the required field at the entrance to the nanopore would be excessive. Hence, there are additional grounds for allowing Claim 15.

Claims 5 and 6 were rejected under 35 U.S.C. 103(a) as being unpatentable over Bohn, in view of Deamer, (and Su as applied to claims 1-4, 7-15, 19, and 20 above), and further in view of Lockhart (US 6,344,316 B1) ("Lockhart"). Applicant traverses this rejection.

In making this rejection, the Examiner argues that Bohn, Deamer and Su teach all of the limitations of Claim 5 except for a leader that is attached to the biopolymer for threading the biopolymer through the nanopore. The Examiner looks to Lockhart as teaching a leader in the form of an end labeling that is attached to a biopolymer that can be detected optically. According to the Examiner, it would be obvious to include the leader of Lockhart in the biopolymers of the remaining references to optimize detection of the biopolymer as the biopolymer traverses the nanopore.

Claim 5 requires that the leader thread the biopolymer through the nanopore. That is, the leader must go through the nanopore first, drawing the rest of the biopolymer after it. To provide such a function, the leader must have a higher electrophoreic mobility than the rest of the biopolymer. If the leader had a lower mobility, the biopolymer would precede the leader and thread the leader through the nanopore. The Examiner has not pointed to any teaching in Lockhart that the labeling compounds taught therein would provide such a function. Hence, even if the Examiner's motivation for combining the various teachings is correct, the Examiner has still not shown that all of the limitations of Claim 5 are met. Accordingly, Applicant submits that the Examiner has not made a prima *facia case* for obviousness with respect to Claim 5, or Claim 6.

Claims 16-18 were rejected under 35 U.S.C. 103(a) as being unpatentable over Bohn in view of Deamer, Su, Lockhart, Griffiths et al. (US 6,770,182 B1) ("Griffiths"), and Sebastin et al. ("Kramers problem for a polymer in a double well," Physical Review E, vol. 62, no. 1, pp. 927-939, July 2000) ("Sebastin") and Han et al. ("Entropic trapping and Escape of Long DNA Molecules at Submicron Size Constriction," Physical Review of Letters, vol. 83, no. 8, 23 August 199, pp. 1688-1689) ("Han"). Applicant submits that Claims 16 –18 as amended above are not obvious in view of the cited prior art.

All of the claims in question require that the electric fields are setup such that the biopolymer moves past the nanopore before being threaded into the nanopore. The Examiner admits that the cited prior art does not explicitly teach this limitation. The Examiner attempts to overcome this lack of teaching by arguing that, statistically, some of the biopolymers must move past the nanopore prior to being threaded into the nanopore. The Examiner's argument is based on the fact that a band in a conventional electrophoresis channel is of finite width and has a distribution. This argument assumes that the biopolymers in the front of the band overshoot the nanopore rather than going directly into the nanopore. The Examiner has not presented any evidence to support this conjecture. At best, the Examiner argues that a biopolymer could go through the nanopore sideways or as a hairpin structure. In particular, the Examiner points to Figure 5 of Sebastin as teaching a hairpin crossing. The problem with the Examiner's argument is that Sebastin does not teach how the hairpin structure is formed. A long molecule can form a hairpin structure in an electrophoresis channel if the portion of the molecule near the bend in the structure is subjected to a greater force than the portions on either side due to the chemical composition of the polymer in the vicinity of the bend. This is the case even in the absence of a nanopore that has an electric field at its opening. The hairpin structure can then thread through the nanopore without ever passing the nanopore. Hence, Applicant submits the Examiner has not shown that some of the biopolymers in the device taught by Bohn pass the nanopore prior to being threaded into the nanopore.

Claims 16 and 18 further require that the leader molecule of the biopolymer enters the nanopore first, under the influence of an electric field, and thus draws the rest of the biopolymer through the nanopore behind it. This is clearly explained in the specification of the current invention in the paragraph beginning on Page 12 line 25. Applicant submits that none of the cited prior art, alone or in combination, discloses this method of threading a biopolymer through a nanopore. As noted above, there is no teaching in the art cited by the Examiner that the leaders identified by the Examiner have this property. Hence, there are additional grounds for allowing Claims 16 and 18.

Claims 1-4, 7-15, 19, and 20 were rejected under 35 U.S.C. 103(a) as being unpatentable over Yamakawa et al. (US 6,806,543 B2) ("Yamakawa"), in view of Bohn, Deamer, and Su. Applicant traverses the rejection of Claim 2. Applicant submits that as currently amended the other Claims are not obvious in view of the cited prior art.

Claim 1 requires a set of electrodes in each of the two channels. Claim 1 has been rewritten to make it clear that the electrodes in the second channel (the channel into which the biopolymer passes after passage through the nanopore) are disposed within the second channel on opposite sides of the nanopore.

The Examiner states that it is **implied** by Column 7, lines 58-65 of Yamakawa that the system taught by Yamakawa has "an electrode for creating electrophoretic movement of the molecule in a second direction (implied by col. 07:58-65, which discloses generating flow of fluid and molecules through the channels by known electrokinetic or electrosmotic methods, which commonly involve applying an electrical field across a pair of electrodes separated by the length of the channel." The Examiner also cites Column 5 line 61 to Column 6 line 5 of Bohn as disclosing "an apparatus substantially the same as claimed including electrodes at the end of the first channel and **implicitly at the end of the second channel, for first and second dimension electrophoresis separations**". Applicant must disagree with the Examiner's assumption that the implied electrodes are in the channels. The electrodes could be at any point that is

electrically connected to the fluid flowing in the channels.

Further, Applicant submits that the passage in Yamakawa cited by the Examiner simply lists a number of possible methods for generating fluid flow, one of the many listed being "electric field induced". The Examiner has not pointed to any teaching regarding the use of any of these methods for driving the movement of molecules of interest through the porous membrane taught by Yamakawa. Applicant submits that the cited passage, in the context of the rest of the paragraph and of the whole system description given by Yamakawa, implies at most that such methods may be used for generating flow along the channel, not in the second direction required by the Claim. Indeed, the Examiner states that such methods involve applying the field "across electrodes separated by the length of the channel", implying that the desired motion is directed along the channel, not out of it.

In addition, even if the Examiner's suggestion were taken that the passage in Yamakawa implies the provision of an electrode "for creating electrophoretic movement", the field could be provided by a set of electrodes that do not satisfy the limitations of the claims in question, and hence, the limitation is not inherently taught in the references.

Finally, as noted above, Applicant submits that electrophoretic motion of molecules passing through the pores in the system taught by Bohn must occur anyway, under the influence of the field that must exist in the second channel to cause the passage through the pores. Hence, Applicant submits that there would be no need to add a second electrode to the system taught by Bohn to achieve electrophoretic separation, since the electrodes taught in Bohn already provide this function. Moreover, even if one were to make that addition, there would be no reason to position that second electrode on the opposite side of the nanopore in the second channel to the position of the first electrode in the second channel. Hence, Applicant submits that the motivation suggested by the Examiner for modifying Bohn by providing a second electrode is flawed

As noted above with respect to the previous rejection of Claim 1, the purpose and function of the set of electrodes in the second channel is to create the electric field that draws the biopolymer, headed by its leader molecule, through the nanopore from the first channel into the second channel. As discussed further (Page 15, lines 17-29), the electric field set up in the second channel can be controlled independently of the field set up in the first channel, and the two fields can be adjusted to manipulate the biopolymer as it traverses the nanopore, stretching or compressing the biopolymer as required in the course of the sequencing operation which is the larger purpose driving this invention. Applicant submits that the use of multiple electrodes, disposed on either side of the nanopore in the second channel, is particularly advantageous in allowing the electric potential at the base of the nanopore to be set and maintained at a desired value, or adjusted as necessary, independent of the significant changes in the resistance of the nanopore as the biopolymer passes through it. Hence, Applicant submits that Claim 1 and the Claims dependent therefrom are not obvious in view of the cited prior art.

Claim 2 requires a substrate having a channel wall and a nanopore in the channel wall. The Examiner points to Yamakawa as teaching a nanopore (110) in the wall of the first microfluidic channel (Figures 1a, 1c, and 1f; and col. 03:29-41; and col. 05:25-33). Applicant submits that Yamakawa teaches a porous membrane 110, separate from the elements containing the channels, and that the pores do not exist in a channel wall that exists within a substrate, as required by the Claim. Bohn, Deamer and Su do not provide the missing teachings. The above amendments to Claim 2 make it clear that the nanopore is through a channel wall that is etched in a surface of the substrate. Hence, Applicant submits that the Examiner has not made a *prima facie* case for obviousness with respect to Claim 2 as amended above.

Claim 3 requires at least one set of electrodes for moving the biopolymer in a second direction through the nanopore after the biopolymer has been moved past the nanopore. Claim 3 has been rewritten to make it clear that the electrodes that create the field to

achieve the motion of the biopolymer in the second direction are located on opposite sides of the nanopore exit. It should also be noted that Claim 3 requires the electrodes to be in the first channel.

The Examiner states that it is implied by Column 7, lines 58-65 of Yamakawa that the system taught by Yamakawa has an electrode for creating electrophoretic movement of the molecule in a second direction (implied by col. 07:58-65, which discloses generating a flow of fluid and molecules through the channels by known electrokinetic or electrosmotic methods, which commonly involve applying an electrical field across a pair of electrodes separated by the length of the channel. Applicant respectfully disagrees. Yamakawa teaches an electrode on each side of the micropore containing sheet to measure the electrical properties of the membrane in the embodiment shown in Figure 10. Such electrodes provide a means for measuring or moving material through the membrane but not along the channel. Yamakawa also teaches moving molecules by electric field induced motion; however, Yamakawa does not specify where the electrodes are that induce such motion. The Examiner attempts to overcome this lack of explicit teaching by looking to the passage starting at column 5, line 61 of Bohn as implicitly teaching electrodes at the end of the channels. While the cited passage describes a potential difference between ends of the channels, it does not state the location of the electrodes that create that potential difference. Such electrodes could, for example, be in the reservoirs that feed the liquid to and from the channels. In which case, the electrodes would not be in the channel as required by Claim 3. For a limitation to be implicitly met, the limitation must always be true, not merely possible or likely. Hence, Applicant submits that Claim 3, and the claims dependent therefrom are not obvious in view of the cited references.

Claim 14 depends from Claim 1 and further requires that the first channel diameter is from 1 to 10 micrometers. The Examiner states that "since the apparatus is a microfluidic apparatus barring a contrary showing the claimed channel dimensions are just a matter of scaling the channel for the expected volume of fluid to be contained by the channel, which will be proportional to the expected sample volume". Applicant

submits that to the contrary, the specific dimensional range specified by Claim 14 is not merely a matter of scaling for the expected fluid volume, but is chosen with regard to other significant criteria.

Specifically, as the electric field drawing the biopolymer of interest through the nanopore is only experienced in the near vicinity of the pore itself, almost all of the fluid present across the diameter of a channel is orders of magnitude greater than the pore diameter that would pass that pore without experiencing that field. Hence, there is a significant advantage in limiting the channel diameter to values large enough to be easily fabricated but small enough to avoid wasting the fluid passed through the device but not subjected to that second field. Hence, Applicant submits that there are additional grounds for allowing Claim 14.

Claims 5 and 6 were rejected under 35 U.S.C. 103(a) as being unpatentable over Yamakawa, in view of Bohn, Deamer, and Su as applied to claims 1-4, 7-15, 19, and 20 above, and further in view of Lockhart. Applicant submits that as dependent on the currently amended Claim 3, Claims 5 and 6 are not obvious over the cited prior art.

With respect to Claim 5, the Examiner argues that Yamakawa as modified by Deamer and Sue teaches all of the limitations of the claim accept for a leader molecule that threads the biopolymer through the nanopore. The Examiner then looks to Lockhart for the missing teaching. Applicant submits that Claim 5 as amended is not obvious in view of the cited art.

First, as noted above, Yamakawa as modified by Deamer and Sue does not teach the limitations of Claim 3. Second, Claim 5 requires that the leader thread the biopolymer through the nanopore. That is, the leader must go through the nanopore first, drawing the rest of the biopolymer after it. To provide such a function, the leader must have a higher electrophoreic mobility than the rest of the biopolymer. If the leader had a lower mobility, the biopolymer would precede the leader and thread the leader through the nanopore. The

Examiner has not pointed to any teaching in Lockhart that the labeling compounds taught therein would provide such a function. Hence, even if the Examiner's motivation for combining the various teachings is correct, the Examiner has still not shown that all of the limitations of Claim 5 are met. Accordingly, Applicant submits that the Examiner has not made a *prima facia* case for obviousness with respect to Claim 5, or Claim 6.

Claims 16 and 18 were rejected under 35 U.S.C. 103(a) as being unpatentable over Yamakawa, in view of Bohn, Deamer, Su, Lockhart, Griffiths, and Sebastin et al. ("Kramers problem for a polymer in a double well," Physical Review E, vol. 62, no. 1, pp. 927-939, July 2000) ("Sebastin") and Han et al. ("Entropic trapping and Escape of Long DNA Molecules at Submicron Size Constriction," Physical Review of Letters, vol. 83, no. 8, 23 August 199, pp. 1688-1689) ("Han"). Applicant submits that Claims 16 and 18 as currently amended are not obvious in view of the cited prior art. Applicant submits that as dependent on the currently amended Claim 1, Claim 17 is not obvious in view of the cited prior art.

All of the claims in question require that the electric fields are setup such that the biopolymer moves past the nanopore before being threaded into the nanopore. The Examiner admits that the cited prior art does not explicitly teach this limitation. The Examiner attempts to overcome this lack of teaching by arguing that, statistically, some of the biopolymers must move past the nanopore prior to being threaded into the nanopore. The Examiner's argument is based on the fact that a band in a conventional electrophoresis channel is of finite width and has a distribution. This argument assumes that the biopolymers in the front of the band overshoot the nanopore rather than going directly into the nanopore. The Examiner has not presented any evidence to support this conjecture. At best, the Examiner argues that a biopolymer could go through the nanopore sideways or as a hairpin structure. In particular, the Examiner points to Figure 5 of Sebastin as teaching a hairpin crossing. The problem with the Examiner's argument is that Sebastin does not teach how the hairpin structure is formed. A long molecule can form a hairpin structure in an electrophoresis channel if the portion of the molecule near the bend in the structure is subjected to a greater force than the portions on either side due to chemical

composition of the polymer in the vicinity of the bend. This is the case even in the absence of a nanopore that has an electric field at its opening. The hairpin structure can then thread through the nanopore without ever passing the nanopore. Hence, Applicant submits that the Examiner has not shown that some of the biopolymers in the device taught by Bohn pass the nanopore prior to being threaded into the nanopore.

Claims 16 and 18 further require that the leader molecule of the biopolymer enters the nanopore first, under the influence of an electric field, and thus draws the rest of the biopolymer through the nanopore behind it. This is clearly explained in the specification of the current invention in the paragraph beginning on Page 12 line 25. Applicant submits that none of the cited prior art, alone, or in combination, discloses this method of threading a biopolymer through a nanopore. As noted above, there is no teaching in the art cited by the Examiner that the leaders identified by the Examiner have this property. Hence, there are additional grounds for allowing Claims 16 and 18.

Respectfully Submitted,

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